Amendments to Drawing Figures

The figures have been amended to correct typographical errors; specifically, the figure labels have been changed from "Figure" to "FIG." as shown in the Annotated Marked-up Drawings. No other changes have been made to the figures. No new matter has been added.

Amendments to the Specification

Please amend the specification as follows. Only typographical errors have been fixed in the following amendments. No new matter has been added herein.

Please amend the specification on page 2, at the paragraph of section [0006], with the following replacement paragraph:

FIG. 1 illustrates the formation of topoisomerase-activated adaptors from synthetic oligonucleotides. Sequences for T7TOPO (SEQ ID NO: 1) and ASTOPO (SEQ ID NO: 2) are shown.

Please amend the specification on page 13, at the paragraph of section [0058], with the following replacement paragraph:

Functionalities encoded by the oligonucleotide adaptor sequences of the invention include promoter sequences, enhancer sequences, transcription initiation sequences, transcription termination sequences, polyadenylation signals, intronic sequences, translation initiation sequences, epitope tag sequences, integration-promoting factor sequences, an mRNA stability-regulating sequence, restriction endonuclease recognition/cleavage sequences, synthetic multiple cloning site sequences, cellular localization encoding sequences, and sites for the covalent or noncovalent attachment of a biological or chemical functional group (as described above). For example, exemplary promoter sequences include phage, viral, prokaryotic and eukaryotic promoter elements. Preferred prokaryotic phage promoter elements include lambda phage promoters (e.g. P[[.sub.R]]$_R$M and P[[.sub.R]]$_R$), T7 phage promoter sequences (e.g. TAATACGACTCACTATA; SEQ ID NO:9), T3 phage promoter sequences (e.g. TTATTAACCCTCACTAAAGGGAAG; SEQ ID NO:10), and SP6 phage promoter sequences (e.g. ATTTAGGTGACACTATAGAATAC; SEQ ID NO: 11). Preferred prokaryotic promoter elements include those carrying optimal -35 and -10 (Pribnow box) sequences for transcription by a prokaryotic (e.g. E. coli) RNA polymerase. In addition, some prokaryotic promoters contain overlapping binding sites for regulatory repressors (e.g. the Lac promoter and the synthetic TAC

promoter, which contain overlapping binding sites for lac repressor thereby conferring inducibility by the substrate homolog IPTG). Prokaryotic genes from which suitable promoters sequences may be obtained include the E. coli lac, ara and trp genes. Preferred eukaryotic promoter sequences include eukaryotic viral gene promoters such as those of the SV40 promoter, the herpes simplex thymidine kinase promoter, as well as any of the various retroviral LTR promoter elements (e.g. the MMTV LTR).

Please amend the specification on page 17, at the paragraph of section [0071], with the following replacement paragraph:

Another aspect of the invention provides a nucleic acid which hybridizes under stringent conditions to a specified nucleic acid. Appropriate stringency conditions which promote DNA hybridization, for example, 6.0.times. sodium chloride/sodium citrate (SSC) at about 45[[ ]]°C, followed by a wash of 2.0.times. SSC at 50[[ ]]°C, are known to those skilled in the art or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6 or in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0.times. SSC at 50[[ ]]°C to a high stringency of about 0.2.times. SSC at 50[[ ]]°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22[[ ]]°C, to high stringency conditions at about 65[[ ]]°C. Both temperature and salt may be varied, or temperature and salt concentration may be held constant while the other variable is changed. In a preferred embodiment, a nucleic acid of the present invention will bind to a vertebrate cDNA nucleic acid sequence or complement thereof under moderately stringent conditions, for example at about 2.0.times. SSC and about 40[[ ]]°C.

Please amend the specification on pages 17 to 18, at the paragraph of section [0074], with the following replacement paragraph:

Standard adaptors may be designed for any particular application. In this example, we prepared universal adaptors for incorporation of a T7 RNA polymerase promoter onto a PCR product. The adaptor preparation starts by hybridization of two synthetic oligonucleotides. As

shown in FIG. 1, the sequence of the first oligonucleotide is 5'-TAATACGACTCACTATAGGGACCCTTGGTGCACCA-3 (T7TOPO; SEQ ID NO[[.]]: 1)'; and the sequence of the second oligonucleotide is 5'-AGGGTCCCTAT-3' (ASTOPO; SEQ ID NO[[.]]: 2). The structure of the oligonucleotides allows them to hybridize with formation of two topoisomerase I recognition sites within one hybrid. DNA hybrids were created by combining equimolar amounts of the T7TOPO (SEQ ID NO: 1) and the ASTOPO (SEQ ID NO: 2) oligonucleotides at 65[[ ]]°C, followed by slow cooling of the mixture to 25[[ ]]°C at a rate of about 0.5[[ ]]°C/minute. Hybridization forms a stable complex of oligonucleotides with two recognition sites within the DNA duplex (FIG. 1). The existence of two nicks in the double strand hybrid does not affect the ability of the topoisomerase activity to recognize, cleave and form a covalent activated intermediate with the T7TOPO (SEQ ID NO: 1) oligonucleotide strand (FIG. 1). This complex was found to be stable for weeks when stored in 50% glycerol at -20[[ ]]°C.

Please amend the specification on page 18, at the paragraph of section [0075], with the following replacement paragraph:

Adaptor activation was performed by incubation of 8 pmol hybrid DNA with 5 units of vaccinia virus topoisomerase I (Epicentre) at 37[[ ]]°C for 15 minutes. Next, PCR products generated from genomic DNA and single-stranded cDNA were generated as target nucleic acids for incorporation of a T7 promoter sequences using the topoisomerase activated adaptors. Two oligonucleotides, corresponding to sense and antisense sequences of the human PRL-1 gene were used to amplify a 483 bp fragment of the gene from human genomic DNA. The PRL-1 gene encodes a protein tyrosine phosphatase present in regenerating liver which is also expressed in foveal cells of the human retina. The sense oligonucleotide corresponded to positions 10021-10041 of the PRL-1 gene and had the sequence GAAGCACATGTCTTTAATGTC (SEQ ID NO[[.]]: 3), while the antisense oligonucleotide corresponded to positions 100503-100481 of the PRL-1 gene and had the sequence GAACTAACATTAATACACATCAC (SEQ ID NO[[.]]: 4). Based on the sequences of human red and green cone pigment cDNAs, sense (GTACCACCTCACCAGTGTCT, SEQ ID NO[[.]]: 5) and antisense (AAATGATGGCCAGAGACCA, SEQ ID NO[[.]]: 6) primers, corresponding to positions 156-

176 and 443-423 of the red/green cone pigment cDNA respectively, were used to generate a 288 bp PCR product from monkey oligo(dT)-primed first strand cDNA.

Please amend the specification on page 20, at the paragraph of section [0084], with the following replacement paragraph:

The longer oligonucleotide may be adapted to carry any useful sequence such as an RNA polymerase promoter sequence at the 5'-end in addition to a recognition site for vaccinia virus topoisomerase I (CCCTT) within 10 bases of the 3' end (underlined sequence in FIG. 1). The 3'-end oligonucleotide also performs two other functions--i.e., it forms duplex DNA downstream of the recognition site and defines specificity for acceptor DNA which has either blunt ends (e.g. PCR products generated with proofreading DNA polymerase) or 3' A overhangs (e.g. PCR products generated with Taq DNA polymerase). The shorter oligonucleotide should be designed to be complementary to the longer one at the toposiomerase I recognition sequence (i.e. 5'-AGGG-3', which is complementary to 5'-CCCT-3' of the T7TOPO (SEQ ID NO: 1) oligonucleotide) as well as an additional few nucleotides of upstream sequence (i.e. 5'-TCCCTAT'3', which is complementary to 5'-ATAGGGA-3' in the T7TOPO (SEQ ID NO: 1) oligonucleotide). Upon hybridization the oligonucleotides form double-stranded DNA upstream and at the topoisomerase recognition site. Moreover, if the oligonucleotides are designed for acceptor DNA with 3' A overhangs, it should be shorter by one base providing complementarity to the first four bases of the recognition site. Topoisomerase I cleaves the DNA at the recognition site forming a covalent bond with the 3'-phosphate at the incised strand. Heterologous acceptor DNA may be covalently bound through the 3'-end phosphodiester bond instead of the cleaved fragment if the following requirements are met: the acceptor DNA is longer than 12 base pairs, the acceptor DNA has 3'-A overhangs, and the acceptor DNA has 5'-dephosphorylated ends.

Please amend the specification on page 21, at the paragraph of section [0087], with the following replacement paragraph:

Vectors for the expression of vaccinia virus topoisomerase I may be generated using standard cloning methods (see e.g. Sambrook et al. (1989) Molecular Cloning: A Laboratory

Manual Cold Spring Harbor Press). The amino acid sequence of vaccinia topoisomerase I (SEQ ID No[[.]]: 8) and the nucleic acid sequence which encodes it (SEQ ID No[[.]]: 7; GenBank Accession No. LI 3447) are shown below.

Please amend the specification on page 9, at the paragraph of section [0088], with the following replacement paragraph:

Vaccinia Topoisomerase I Protein Sequence (SEQ ID NO: 8):

Please amend the specification on page 10, at the paragraph of section [0089], with the following replacement paragraph:

Vaccinia Topoisomerase I Gene, Nucleotide Sequence (SEQ ID NO: 7):